Program/Abstract #381
Spinal cord regeneration in *Xenopus laevis* proceeds through activation of Sox2+ cells
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Xenopus laevis tadpoles have the capacity to regenerate their spinal cord after an injury; however, the mechanisms involved in this process are unknown. We hypothesize that neural progenitors are necessary for spinal cord regeneration. In order to evaluate that, we have studied Sox2 function, a neural progenitor marker, during spinal cord regeneration. We found that Sox2 expression was upregulated after tail amputation and spinal cord transection, two different models of spinal cord injury. Additionally, we observed Sox2+ cells undergoing proliferation and LRC increase in response to tail amputation. The activation of neural progenitors suggests a role for ependymal regrowth and neurogenesis in *X. laevis* CNS regeneration. We also analyzed the role of neural progenitors during spinal cord regeneration by the overexpression of a dominant negative form of Sox2 after amputation. Experimental reduction of Sox2 activity diminished proliferation of spinal cord resident cells and impairs tail regeneration. Furthermore, Sox2 expression levels are correlated with functional recovery after transection at different stages of metamorphosis. We concluded that Sox2+ cells are necessary for spinal cord and tail regeneration and lead to a model whereby spinal cord damage activates tissue specific progenitor proliferation.

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Program/Abstract #382
miRNAS and regulation of retinoid signaling in the regenerating adult newt spinal cord
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Appendage regeneration is a complex process unique to only a few species of adult vertebrates, including the Urodele amphibians. One current focus involves the identification of molecules and signaling pathways that control the formation of the tail blastema, as well as the outgrowth and patterning of the regenerating spinal cord. Recent efforts in our lab have centered on defining the upstream and downstream regulators of retinoid signaling that result in the production of a functional caudal spinal cord after tail amputation. We have previously demonstrated that inhibition of retinoid signaling through the RARβ2 receptor using a specific antagonist, LE135, inhibits the outgrowth of the ependymal tube during the first 6 days after tail amputation. We now demonstrate that miR133a is significantly down regulated in the ependymal tube during this same period. Our most recent results with microRNA-based profiling have identified at least 18 highly conserved miRNAs that display significant changes in expression in tail regenerates treated with LE-135 compared to DMSO treated control regenerates. An analysis of the expression patterns and putative functions of these potential regulators of retinoid signaling are underway.

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Program/Abstract #383
The homology of EVI5 and ABK sequences among animals
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Amphibian Axolotl (*Ambystoma mexicanum*) can regenerate its limbs after amputation. During the early stage of regeneration, a G2 maintaining protein, ecotropic viral integration site 5 (EVI5), has dramatically increased, which suggests the delay of cells entering mitosis. Aurora B Kinase (ABK) assists to degrade EVI5, which moves cells from G2 to mitosis. Unfortunately, sequence information of EVI5 and ABK was unknown for axolotl, hindering future study on their roles in axolotl limb regeneration. The cDNA sequences of ABK and EVI5 from human, mouse, rat, and xenopus were then collected from GenBank database and compared utilizing ClustalW software. Total RNA was extracted from axolotl tissue for degenerate reverse transcriptase polymerase chain reaction (RT-PCR). GADPH was used as an internal control. The RT-PCR products were analyzed by two direction sequencing. After comparison, 342 bp of nucleotides from EVI5 and 1035 bp from ABK were highly conservative among animals and used as templates to design primers for degenerate RT-PCR. The sequencing analysis on axolotl RT-PCR products identified 166 bp and 261 bp nucleotides for EVI5 and ABK, respectively. This study provided us the partial sequence information of axolotl EVI5 and ABK, which advanced the study on their roles in regeneration.

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Program/Abstract #384
Myelinated peripheral axons in the adult zebrafish maxillary barbel (ZMB): A new model for adult re-myelination during sensory appendage regeneration
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Myelination is a cellular adaptation allowing saltatory conduction along axons. Adult zebrafish can regenerate myelin in the central nervous system (CNS), lateral line, and optic nerve. We have investigated the regeneration of peripheral nervous system (PNS) axons in the zebrafish maxillary barbel (ZMB), an external and optically clear sensory appendage. The ZMB is innervated by axons descending from the facial nerve; after amputation, damaged axons regrow into the regenerating barbel shaft and make connections to epithelial taste buds (LeClair & Topczewski, 2010). We sought to determine whether ZMB axons are myelinated, and, if so, whether myelination is restored during regeneration. Myelin was detected using histological stains, immunohistochemistry and transmission electron microscopy (TEM). Each ZMB contains approximately 100 small axons (avg. diameter $<2\,\mu m$); more than 90% are thickly myelinated. Two myelin-specific transcripts, myelin basic protein (MBP) and myelin protein zero (MPZ), were confirmed by RT-PCR. Regenerating ZMB axons are initially unmyelinated or have a few loose myelin layers; regeneration in these appendages was followed for >2 months to assess axon diameter, myelin thickness and organization, and the presence of Schwann cells. Finally, we examine if the regenerating ZMB expresses conserved members of the pro-myelinating gene network, including sox10, oct6 (=pou3f1) and krox20a/b (=egr2a/b). These observations extend our understanding of myelinated fibers in the adult zebrafish PNS, and demonstrate the potential of the ZMB as a novel system in which to study axon regeneration, Schwann cell migration and re-myelination after adult injury. Supported by R15HD064169 (to EEL).

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